

Rec'd PCT/PTO 08 JUL 2004

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
24 July 2003 (24.07.2003)

PCT

(10) International Publication Number  
WO 03/059322 A1(51) International Patent Classification<sup>7</sup>: A61K 9/127,  
C12N 15/88, A01N 43/04SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC,  
VN, YU, ZA, ZM, ZW.

(21) International Application Number: PCT/US03/00380

(22) International Filing Date: 8 January 2003 (08.01.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/346,284 9 January 2002 (09.01.2002) US(71) Applicant (for all designated States except US): ELAN  
PHARMACEUTICALS, INC. [US/US]; 800 Gateway  
Boulevard, South San Francisco, CA 94080 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): TONG, Shangguan  
[CN/US]; 17 Taft Court, Princeton, NJ 08540 (US). LI,  
Xingong [CN/US]; 103 Princeton Arms N., Cranbury,  
NJ 08512 (US). MEERS, Paul, R. [US/US]; 29 Berrien  
Avenue, Princeton, NJ 08550 (US). PERKINS, Walter, R.  
[US/US]; 248 Federal City Road, Pennington, NJ 08534  
(US). POLOZOVA, Alla [CA/US]; Orchard 105M, 60  
One Mile Road, Cranbury, NJ 08512 (US).(74) Agents: REA, Teresa, Stanek et al.; Burns, Doane,  
Swecker & Mathis, L.L.P., PO Box 1404, Alexandria, VA  
22313-1404 (US).(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,  
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,  
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,(84) Designated States (regional): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),  
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,  
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI,  
SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN,  
GQ, GW, ML, MR, NE, SN, TD, TG).

## Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations

## Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: EFFICIENT NUCLEIC ACID ENCAPSULATION INTO MEDIUM SIZED LIPOSOMES

(57) Abstract: A method for preparing liposomes containing at least one nucleic acid encapsulated therein comprising the following steps: (A) mixing a gel or a liquid containing gel particles with aqueous medium Z1 to directly form the liposomes containing the at least one nucleic acid encapsulated therein; (B) (i) mixing a gel or a liquid containing gel particles with aqueous medium Z1 to form a curd or curdy substance; and (ii) mixing the curd or curdy substance with aqueous medium Z2 to directly form the liposomes containing the at least one nucleic acid encapsulated therein; or (C) (i) cooling a gel or a liquid containing gel particles to form a waxy substance; and (ii) mixing the waxy substance with aqueous medium Z1 to directly form the liposomes containing the at least one nucleic acid encapsulated therein; wherein said gel or liquid containing gel particles comprises at least one liposome-forming lipid, at least one fusogenic lipid, a water-miscible organic solvent and the at least one nucleic acid; wherein an amount of the at least one fusogenic lipid is at least 20% by weight of a lipid content of the gel or the liquid containing gel particles; and wherein the aqueous media Z1 and Z2 are the same or different.

WO 03/059322 A1